Bioremediation of Soil Contaminated with Petroleum Hydrocarbons Collected From Arid and Semi- Arid Regions of Rajasthan

A Synopsis

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INTRODUCTION

Hydrocarbon contamination

One of the major environmental problems today is hydrocarbon contamination resulting from the activities related to the petrochemical industry. Accidental releases of petroleum products are of particular concern in the environment. Hydrocarbon components have been known to belong to the family of carcinogens and neurotoxic organic pollutants. Currently accepted disposal methods of incineration or burial insecure landfills can become prohibitively expensive when amounts of contaminants are large. Mechanical and chemical methods generally used to remove hydrocarbons from contaminated sites have limited effectiveness and can be expensive. Bioremediation is the promising technology for the treatment of these contaminated sites since it is cost-effective and will lead to complete mineralization. Bioremediation functions basically on biodegradation, which may refer to complete mineralization of organic contaminants into carbon dioxide, water, inorganic compounds, and cell protein or transformation of complex organic contaminants to other simpler organic compounds by biological agents like microorganisms (Das and Preethy, 2010).

With an ever increasing world's population, there is a concomitant increase in the demand for petroleum and petroleum products, which apparently constitutes a source of environmental pollution (Raven *et al.,* 1993). Oil pollution is a major environmental concern in many countries, and this has led to a concerted effort in studying the feasibility of using oil-degrading bacteria for bioremediation (Akoachere *et al*., 2008).

Petroleum-based products are the major source of energy for industry and daily life. Leaks and accidental spills occur regularly during the exploration, production, refining, transport, and storage of petroleum and petroleum products. The amount of natural crude oil seepage was estimated to be 600,000 metric tons per year with a range of uncertainty of 200,000 metric tons per year (Kvenvolden and Cooper, 2003). The technology commonly used for the soil remediation includes mechanical, burying, evaporation, dispersion, and washing. Environmental pollution with petroleum and petroleum products (complex mixture of hydrocarbons) has been recognized as one of the most serious current problems especially when associated with accidental spills on large-scale. If this occurs, hydrocarbons may reach the water table before becoming immobilized in the soil. They spread horizontally on the ground water surface and continue to partition into ground water, soil pore space, air and to the surface of soil particles. The vast range of substrates and metabolites present in hydrocarbon

impacted Soils surely provide an environment for the development of a quite complex microbial community (Butier and Mason, 1997).

Biodegradation

Biodegradation is the chemical dissolution of materials by bacteria or other biological means. The term is often used in relation to ecology, waste management, biomedicine, and the natural environment bioremediation and is now commonly associated with environmentally friendly products that are capable of decomposing back into natural elements. Organic material can be degraded aerobically with oxygen, or anaerobically, without oxygen. A term related to biodegradation is biomineralisation, in which organic matter is converted into minerals. Biosurfactant, an extracellular surfactant secreted by microorganisms, enhances the biodegradation process.Some microorganisms have a naturally occurring, microbial catabolic diversity to degrade, transform or accumulate a huge range of compounds including hydrocarbons (e.g. oil), polychlorinated biphenyls (PCBs), polyaromatic hydrocarbons (PAHs), pharmaceutical substances, radionuclides and metals. Major methodological breakthroughs in microbial biodegradation have enabled detailed genomic, metagenomic, proteomic, bioinformatic and other high-throughput analyses of environmentally relevant microorganisms providing unprecedented insights into key biodegradative pathways and the ability of microorganisms to adapt to changing environmental conditions(wikipedia.org).

OBJECTIVES

1. Collection of soil sample from arid (Barmer) and semi-arid (Jaipur-Ajmer) regions of Rajasthan.
2. Physico-chemical characterization of collected soil samples.
3. Isolation and identification of microorganism from collected soil sample.
4. Biochemical characterization of the isolated Microorganisms.
5. Genetic characterization of the isolated microorganisms.
6. Determination of optimum growth conditions of selected strains for degradation of 2T engine oil.
7. Determination of oil degrading potential of selected strain using gas chromatography / HPLC.

REVIEW OF LITERATURE

The review of literature of present work is divided into two parts-

Work at International level

1. Lubricating oil-degrading bacteria in soils from filling stations and auto- mechanic workshops in Buea, Cameroon: occurrence and characteristics of isolates (South Africa) - Akoachere *et al.,* (2008).

2. Petroleum Hydrocarbon–Degrading Microbial Communities in Beaufort–Chukchi Sea Sediments(Fairbanks)- Braddock *et al*., (2004).

3. Bioremediation of soil heavily contaminated with crude oil and its products: composition of the microbial consortium (Belgrade, Serbia)- Milic *et al.,* (2009).

4. Efficiency of Indigenous and Inoculated Cold-Adapted Soil Microorganisms for Biodegradation of Diesel Oil in Alpine Soils (Austria)- Margesin and Schinner, (1997).

5. Investigation of the bioremediation potential of aerobic zymogenous microorganisms in soil for crude oil biodegradation (Belgrade, Serbia)- Šolević *et al*., (2011).

6. Laboratory evaluation of crude oil biodegradation with commercial or natural microbial inocula1 (Canada) - Thouand *et al*., (1999).

7. Biodegradation of diesel oil in soil by a microbial consortium (México)-Márquez-rocha *et al*., (2001).

8. Isolation of bacteria from mechanic workshops' soil environment contaminated with used engine oil (Nigeria) - Udeani *et al*., (2009).

9. High Performance Phenol Degrading Microorganisms Isolated from Wastewater and Oil-Contaminated Soil (Malaysia)- Mailin and Firdausi, (2006).

10. Presence of opportunistic oil-degrading microorganisms operating at the initial steps of oil extraction and handling (Bellaterra,Spain)- Sánchez *et al*., (2006).

Work at National level

1. Biodegradation of Phenol by Oxygenase Producing Thermophilic Microorganisms (Rajasthan, India)- Mathur *et al*., (2008).

2. Crude petroleum-oil biodegradation efficiency of *Bacillus subtilis* and *Pseudomonas aeruginosa* strains isolated from a petroleum-oil contaminated soil from North-East, India (Tezpur,India)- Das and Mukherjee, (2006).

3. Plasmid Associated Anthracene Degradation by *Pseudomonas* sp. Isolated from Filling Station Site (Punjab, India)- Kumar *et al*., (2010).

4. Characterization of 2T engine oil degrading indigenous bacteria, isolated from high altitude (Mussoorie, India) - Jain *et al*., (2010).

5. Phytoremediation Potential of Some Multipurpose Tree Species of Indian Thar Desert in Oil Contaminated Soil (Rajasthan) - Mathur *et al*.,(2010).

6. Microbial Degradation of Petroleum Hydrocarbon Contaminants: An Overview (Tamil Nadu) - Das and Preethy (2010).

7. Bioremediation of Petroleum Oil Contaminated Soil and Water (Rajasthan) -Jain *et al.*,(2011).

8. Assessment of Genetic Diversity In *Pseudomonas fluorescens* Using PCR- Based Methods (Andhra Pradesh) - Charan *et al.*, (2011).

Methodology

1. Soil samples will be collected from Arid (Barmer) and Semi-Arid (Jaipur-Ajmer) regions of Rajasthan in a sealed polythine bags for two times in a year.

2. Soil texture, moisture content, TDS, electric conductivity, soil salinity, organic carbon content, total nitrogen content, available phosphorus in soil sample, available potassium and sodium, Water Holding Capacity (WHC) of soil, chloride (Cl^-) content and carbonate (Co_3^{2-}) and bicarbonate (HCO_3^-) in soil will be determined to analyze the physicochemical properties of soil**.**

3. For isolation of bacterial colonies Nutrient agar media will be used and for isolation of fungal colonies PDA or SDA will be used. The isolated microorganism will be identified by morphological, culture and biochemical tests. Selected fungal / bacterial isolates will be finalized for bioremediation purpose.

4. The genetic similarities and dissimilarities of isolated strains from different locations of same species will be determined using RAPD markers.

5. The optimum condition (pH, temperature and salt concentration) for growth of microorganisms will be determined.

6. Oil degrading potential at variable temperature and pH will be determined specrophtometrcally.

Laboratory studies on selected components of the 2T engine oil will be carried out in an aqueous medium (pH 7.0). For this purpose MSM (minimal salt medium) supplemented with 1% (v/v) 2T engine oil and washed bacterial culture will be inoculated. An uninoculated control flask will be incubated in parallel to monitor abiotic losses of the 2T engine oil. In the control (abiotic sample), mercuric chloride (10 mg) will be added to ensure that only abiotic conditions prevailed. All the flasks will be incubated at 30ºC for 30 days on incubator shaker (170 rpm). 2T engine oil will be extracted in a soxhlet apparatus with 160 ml of n-hexane and dichloromethane (DCM) (1:1, v/v) for 24 hours. The organic phase will be concentrated by evaporation of the organic solvent after drying over Na_2SO_4, separation and fraction of the different petroleum hydrocarbon achieved from growth medium and analyzed by gas chromatography - mass spectrometry (GC-MS; Geerdink *et al*, 1993).

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